The specification has been amended, as suggested by the Examiner, whose helpful suggestions are acknowledged, with appreciation.

The specification was amended in the Preliminary Amendment of April 28, 2000, to include the Sequence Listing attached thereto. A computer-readable and paper copy of the Sequence Listing were submitted April 28, 2000, along with a Letter indicating that no new matter was added and that the paper and computer-readable copies of the Sequence Listing were the same. A copy of the Preliminary Amendment, Letter and Sequence Listing submitted April 28, 2000, along with the undersigned's postcard receipt from the same and a copy of the Notice to Comply received with the Office Action of January 10, 2001 (Paper No. 6) are also attached. The applicants respectfully submit the application complies with the requirements for patent applications containing nucleotide sequence and/or amino acid sequence disclosures. The Examiner is requested to contact the undersigned however if anything further is required in this regard.

The specification has been amended as suggested by the Examiner and withdrawal of the objection to the same is requested.

The specification has been amended to include a section entitled Brief Description of the Drawings and to include an Abstract, as was published in the parent Application No. PCT/FR98/02320 (published as WO 99/23251).

The Section 101 rejection of claims 11 and 12 is moot in view of the above. The pending claims are submitted to define patentable subject matter.

The Section 112, second paragraph, rejection of claims 1-15 is moot in view of the above. The pending claims are submitted to be definite such that one of ordinary skill in the art would appreciate the metes and bounds of the claimed subject matter.

Specifically, with regard to the Examiner's helpful comments on pages 4-12 of Paper No. 6, the applicants note the following.

The preamble of the amended claims has been revised, as suggested by the Examiner in paragraph 4A. The term "gene rearrangement" will be recognized to encompass different situations involving exchange of genetic material, as described on page 1, for example, of the specification. The claims have been amended with the Examiner's comments in paragraphs 4C through 4G of Paper No. 6 in mind. With regard to the Examiner's comments in paragraph 4H, the applicants note that one primer is specific for the target gene. With regard to the Examiner's comment in 4I, the applicants note the claims have been amended to recite "nucleotide" as suggested by the Examiner. The applicants submit the term "fusion gene" will be recognized by one of ordinary skill in the art, within the context of the present application, to include a target gene having fused with a gene sequence on the genome designated as a "fusion partner", further to a rearrangement, for example, a translocation. With regard to the Examiner's comment at paragraph 4K of Paper No. 6, the applicants note claim 1 has been rewritten as new claim 16 to provide antecedent basis for "PCR products". As for the Examiner's comment in paragraph 4L, the applicants note that the prior recitation of "markers" has now been recited as "probes". The applicants note the claims have been amended to recite "specific for either the target gene or any fusion partner" in place of

the previous recitation objected to by the Examiner in paragraph 4M of Paper No. 6. The applicants submit "a rearrangement" would be understood by one of ordinary skill in the art to include the rearrangement to be detected for a given patient.

*With regard to the Examiner's objection in paragraph 4O of Paper No. 6, the applicants comment that in a particular case, for example of a patient having an acute leukemia, where MLL gene is supposed to be rearranged, and where the fusion partner is to be identified. With the asymmetrical PCR, it is possible to amplify MLL associated with any fusion partner. When using probes specific for known fusion partners for MLL, a hybridization will indicate that the rearrangement did occur with a known fusion partner. On the contrary, in the absence of hybridization, it will be concluded that the rearrangement occurred with a fusion partner which had not been identified at that time. The claims are submitted to be definite in this regard.

With regard to the Examiner's comments in paragraph 4P of Paper No. 6, the previous recitation of "in their whole" means that all the target genes which have fused with a fusion partner will be detected, irrespective of the fusion partner. With regard to the Examiner's comments in paragraphs 4Q-4S of Paper No. 6, the applicants submit the claims have been amended with the Examiner's comments in mind and the pending claims are definite in this regard. Moreover, the claims have been amended to delete references to Tm, as objected to by the Examiner at paragraph 4T of Paper No. 6.

With regard to the Examiner's comments in paragraph 4U of Paper No. 6, the applicants submit that page 6, line 19 and in Example 1.4, for example, a reporter

molecule capable of being further recognized during the detection step is incorporated during the PCR. The pending claims reflect as much.

The Examiner's objection to claims 3-5 stated in paragraph 4V is moot in view of the amended claims.

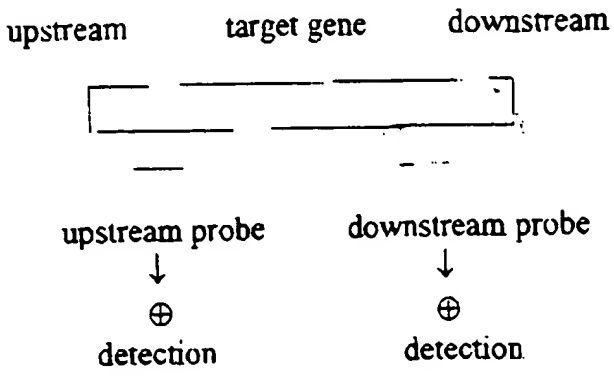
With regard to the Examiner's objections stated in paragraphs 4W and 4X, the applicants note the claims have been amended to recite "labeled" and "complementary" to overcome the previous objection.

With regard to the Examiner's objections stated in paragraph 4Y the applicants note that "fusion partners" is a gene fragment the target gene fuses with, as would be recognized by one of ordinary skill in the art after reviewing the present specification and in view of the generally advanced level of skill in the art at the time of the present invention.

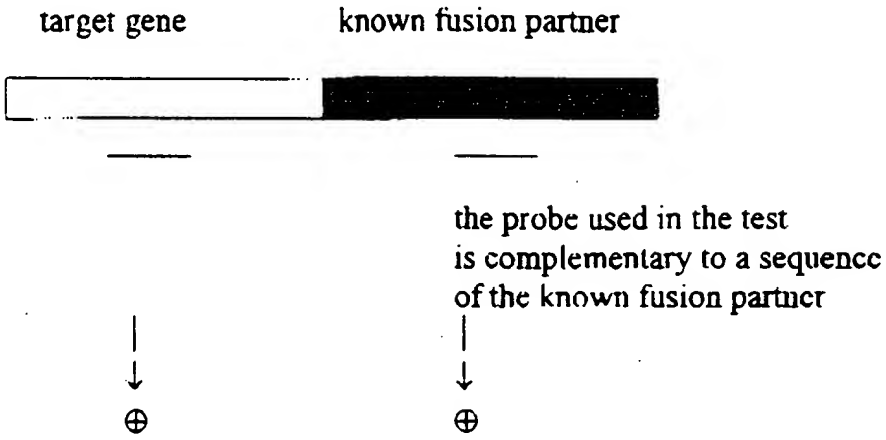
As noted above, antecedent basis for "probes" has been provided in the amended claims, in response to the Examiner's comment in paragraph 4Z. With regard to the Examiner's comment in paragraph 4AA, the claims have been amended to recite "covalently bonded". Moreover, claim 5 has been rewritten as new claim 20 in response to the Examiner's comment in paragraph 4AB. The objected-to "T patterns" has been recited in the amended claims as "T nucleotides". In response to the Examiner's comment in paragraph 4AD, the applicants have amended the objected-to phrase to recite "random repeat of nucleotides". The claims have been amended in response to the Examiner's comments of paragraphs 4AE through 4AL and 4AN.

In response to the Examiner's comment in paragraph 4M, the applicants note the diagnostic test of the present invention may be schematically summarized as follows:

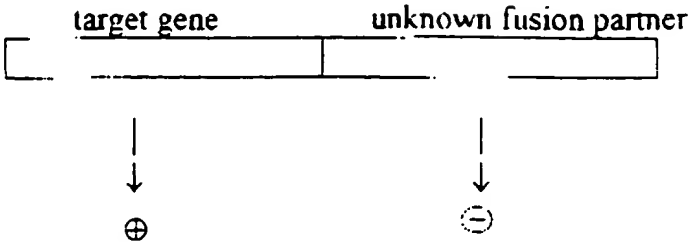
① no rearrangement



② rearrangement with known partner



③ rearrangement with unknown partner



Claims 8 and 9 have been rewritten as new claims 23 and 24 in response to the Examiner's comment in paragraph 4AO. With regard to the Examiner's comment in paragraph 4AP, the previous recitation of "pattern" has been deleted from the claims. Antecedent basis has been provided for the recitation of all oligonucleotide cassette.

The applicants respectfully submit the recitations of "fusion transcripts" and "digoxygenine" will be understood by one of ordinary skill in the art. Specifically, fusion transcripts, for example, refer to the gene fusion products encoded by the patients' cDNa. Digoxygenine is a well known compound which would be recognized by one of ordinary skill in the art.

The previous recitation of "its substrate" has been amended in the rewritten claims as "the enzyme substrate" in response to the Examiner's comment at paragraph 4AU of Paper No. 6. Similarly, the previous recitation of "colored" has not been repeated in the amended claims. The applicants respectfully submit that one of ordinary skill in the art would appreciate that the reactions between the anti-digoxygenine antibody and the enzyme substrate which results in the release of a product which will be detected.

The previous recitation of "should be bonded" objected to by the Examiner at paragraph 4AW has not been repeated in the amended claims. The amended claims are submitted to obviate the objection stated in paragraph 4AX of Paper No. 6. With regard to the Examiner's comments in paragraph 4AY, "the amplification products" have been deleted and the claims now recite "PCR products" with appropriate antecedent basis. The prior recitation of "known partner probes" objected to by the Examiner at

paragraph 4AV has been amended to recite "complementary known partners" in the amended claims. Claim 11 has been rewritten as new claim 28 to provide a recitation of active method steps. The previous recitation of "such as", noted in paragraph 4AAB of Paper No. 6 has not been repeated in the amended claim. Similarly, the objected-to phrases noted in paragraphs 4AAC and 4AAD of Paper No. 6 are moot in view of the above amendments.

The claims are submitted to be definite.

The Section 103 rejection of claims 1-15 over Corral (PNAS (1993) 90:8538-42) in view of Liu (Genomics (1995) 25: 674-681) is moot in view of the above. The pending claims are submitted to be patentable over the cited art and the Examiner is requested to consider the following in this regard.

The applicants respectfully submit Corral discloses chromosomal translocations of MLL gene in acute leukemia. The translocations of Corral were analyzed by Southern blots on patient's genomic DNA or by PCR (or RAP) and sequencing.

The applicants submit however that Southern blots would not have enabled two fragments of the same size to be discriminated and the combination PCR/sequencing, even if useful for research purposes, is totally inappropriate for a commercial diagnostic test due to the labor-intensive nature of the operation (insertion of fragments in plasmids, culture of bacteria).

The applicants submit that Liu et al disclose a TAIL-PCR strategy and the sequencing of the TAIL-PCR products and again Southern blots.

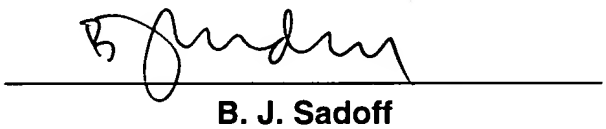
There is no teaching or suggestion however in Corral et al or Liu et al to amplify all target genes and to only detect those which have undertaken a rearrangement using the probes as defined in the pending claims. Such an inventive concept and the means for carrying out the invention are not taught or suggested by the cited documents.

The claims are submitted to be patentable over the combination of cited art.

In view of the above, the pending claims are submitted to be in condition for allowance and a Notice to that affect is requested.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: 

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MARKED UP SPECIFICATION

Page 3a, please delete the paragraph starting on line 19:

"The so-called semi-specific PCR technique employed is performed in such a way as to identify the [jonction] junction fragment in the breakpoint region, and the sequence being then analysed. This article does not then teach [asymetric] asymmetric amplification to amplify the whole fusion genes and to reveal only genes implicated in the rearrangement."

and insert the following therefor:

--The so-called semi-specific PCR technique employed is performed in such a way as to identify the junction fragment in the breakpoint region, and the sequence being then analysed. This article does not then teach asymmetric amplification to amplify the whole fusion genes and to reveal only genes implicated in the rearrangement.

Page 4, between lines 11 and 12, insert the following:

--**BRIEF DESCRIPTION OF THE DRAWINGS**

Figs. 1A, 1B and 1C illustrate the detection protocol of a gene rearrangement with known fusion partners, as described in Example 1.—

Pages 5 and 6, delete the paragraph bridging pages 5 and 6:

"PCR products carry a marker (digoxigenine, biotin or fluorophore for example) by which they will be detected. Such marker is carried by a [desoxynucleotide] deoxynucleotide embodied into the PCR products during the second amplification."

and insert the following therefor:

-- PCR products carry a marker (digoxigenine, biotin or fluorophore for example) by which they will be detected. Such marker is carried by a deoxynucleotide embodied into the PCR products during the second amplification.—

Page 7, delete the paragraph beginning at line 3:

"A detection alternative, to highlight numerous genes rearrangements on a large number of genes, in a single test, is based on the DNA chips technology and comprises using oligonucleotidic or cDNA probes secured to a miniaturized support. Each probe or hybridization unit may advantageously be individually controlled by an electric field. In another alternative, the internal probes are advantageously [immunobilized] immobilized on strips."

and insert the following therefor:

-- A detection alternative, to highlight numerous genes rearrangements on a large number of genes, in a single test, is based on the DNA chips technology and comprises using oligonucleotidic or cDNA probes secured to a miniaturized support. Each probe or hybridization unit may advantageously be individually controlled by an electric field. In another alternative, the internal probes are advantageously immobilized on strips.--

Page 7, delete the paragraph beginning at line 17:

"The internal probes are advantageously [immunobilized] immobilized on strips."

and insert the following therefor:

--The internal probes are advantageously immobilized on strips.—

Pages 8 and 9, delete the paragraph bridging pages 8 and 9:

"According to an advantageous embodiment of the invention, implemented to detect translocations involving the MLL gene, a cDNA pool is synthesized from the RNA extracted from the sample under investigation with the aid of primers including a cassette of about 30 to 35 nucleotides, complemented by a sequence of 6 or 9 random nucleotide patterns, and an anchored PCR is performed using a primer located on the MLL' exon 5, as specific sense primer. Where a second amplification cycle is performed, an internal [sens] sense primer is used to increase the specificity. The random primer is advantageously selected as complementary to the oligonucleotides cassette used on the reverse transcription step."

and insert the following therefor:

-- According to an advantageous embodiment of the invention, implemented to detect translocations involving the MLL gene, a cDNA pool is synthesized from the RNA extracted from the sample under investigation with the aid of primers including a cassette of about 30 to 35 nucleotides, complemented by a sequence of 6 or 9 random nucleotide patterns, and an anchored PCR is performed using a primer located on the MLL' exon 5, as specific sense primer. Where a second amplification cycle is performed, an internal sense primer is used to increase the specificity. The random primer is advantageously selected as complementary to the oligonucleotides cassette used on the reverse transcription step.--

Page 13, delete the paragraph beginning on line 15:

"(1) The cDNAs are synthesized from the total RNAs in the sample studied, by reverse transcription (RT), then (2) the cDNA pool is amplified by PCR and (3) the

transcripts are checked for specificity. [Said steps are illustrated by the schedule given on the single figure.]

and insert the following therefor:

--(1) The cDNAs are synthesized from the total RNAs in the sample studied, by reverse transcription (RT), then (2) the cDNA pool is amplified by PCR and (3) the transcripts are checked for specificity.--

Page 16, delete the paragraph beginning at line 1:

"A second amplification cycle is completed to assess long fragments, using 1 µl of product obtained from the first PCR. An internal [sens] sense primer with respect to the first cycle primer is used with an identical antisens primer; to perform the ELISA detection, the dTTP is replaced by a dTTP + DIG-dUTP^{R} mixture (Boehringer; 1558 706) to the 1 :19 ratio."

and insert the following therefor:

-- A second amplification cycle is completed to assess long fragments, using 1 µl of product obtained from the first PCR. An internal sense primer with respect to the first cycle primer is used with an identical antisens primer; to perform the ELISA detection, the dTTP is replaced by a dTTP + DIG-dUTP^{R} mixture (Boehringer; 1558 706) to the 1 :19 ratio.--

Page 21, delete the paragraph beginning on line 20:

"Amplification is obtained as described in the example 1 using the following [sens] sense primers:"

and insert the following therefor:

-- Amplification is obtained as described in the example 1 using the following sense primers:--.

C10

ABSTRACT OF THE DISCLOSURE

The invention concerns a method for diagnosing in vitro pathologies associated with gene arrangements which consists in subjecting the DNA under study to at least a step of asymmetric amplification with a single pair of primers and in only revealing the gene capable of being involved in gene fusion insofar as the fusion exists. The invention is applicable to the diagnosis of leukaemia.